Human UGT1A9 Microsomes

Catalog Number: 455319 (Old M319A)

Storage Conditions: Store at -80°C

Lot Number: xx

Date Released: Day Month Year

Package Contents: 0.5 ml

Protein Content: 10.0 mg/ml in 100 mM potassium phosphate (pH 7.4)

Cytochrome c Reductase Activity: xx nmole/(min x mg protein)

Cytochrome P450 Content: xx pmole/mg protein

7-Hydroxy-4-trifluoromethylcoumarin Conjugating Activity: xx pmole/(min x mg protein)

xx = Actual values can be found on the data sheet accompanying each shipped product

This activity is catalyzed by UGT1A9 which is expressed from a transfected human UGT1A9 cDNA the cell line used to prepare these microsomes. This activity is not detectable in control cell microsomes (Catalog No. 455101 (Old M101A) or 455102 (Old M101B)).

Method: A 0.25 ml reaction mixture containing 0.16 mg/ml protein, 0.6 mM uridine diphosphoglucuronic acid (UDPGA), 10 mM magnesium chloride, alamethicin (16 ug/ml), and 50 hydroxy-4-trifluoromethylcoumarin in 100 mM tris (pH 7.5) was incubated at 37°C for 10 minute After incubation, 0.05 ml of the reaction was added to 1.0 ml 100 mM potassium phosphate (pH and the fluorescence was determined with excitation at 410 nm and emission at 510 nm in a spectrofluorometer. The activity was quantitated by subtracting the fluorescence of the reaction mixture from the fluorescence of a blank (with substrate and protein, but without UDPGA) and standardized to the fluorescence of 7-hydroxy-4-trifluoromethylcoumarin.

Advice:

- Thaw rapidly in a 37°C water bath. Keep on ice until use. Aliquot to minimize freeze-thawing cycles. The activity of this preparation has been found to be unaffected by up to 5 freeze/th cycles.
- Linearity of the reaction with respect to protein concentration and time of incubation should determined for each substrate/incubation condition examined.
- The UGT activity of this product exhibits the latency typical for microsome preparations. The activity can be increased 2 to 3 fold by the addition of alamethicin (Sigma Chemical Compar Catalog No. A-4665). The optimal alamethicin concentration should be determined for each substrate/incubation condition examined. For the conditions described above, the optimal alamethicin concentration was found to be 4 to 8 ug/ml.
- Production of metabolites by this preparation is monotonic for at least 1 hours using the abc reaction conditions.
- Analysis of UGT1A9 form-specific metabolism should include a concurrent control for the nat UGT activity. GENTEST microsome products, Catalog Numbers 455101 (Old M101A) and 45 (Old M101B), are suitable for control preparations.

U.S. Patent Nos. 4,532,204, 4,686,186 and 5,128,255.

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